

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

App. No. : 10/699,512 Confirmation No. 3570
Applicant : Bennett, G.N.
Filed : October 31, 2003
TC/A.U. : 1637
Examiner : Fredman, J.N.
Docket No. : 31175413-003002
Customer No. : 51738
Entitled : RECOMBINATION ASSEMBLY OF LARGE DNA FRAGMENTS

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF GEORGE N. BENNETT UNDER 37 CFR § 1.131

I, George N. Bennett, Declare as follows:

I am at least 18 years of age and am competent in all respects to make the following statements.

1. I am the sole inventor for claims 1-8 currently pending in US Patent Application No. 10/699,512.
2. The work presented in US Patent Application No. 10/699,512 was conceived prior to December 10, 1999.
3. Although the dates have been redacted, the attached laboratory PowerPoint presentation (Exhibit A) demonstrates the conception or practice of the invention prior to December 10, 1999.
4. Although the dates have been redacted, the attached laboratory notebook (Exhibit B) demonstrates the conception or practice of the invention prior to December 10, 1999.

I further declare that all statements made herein of my own knowledge are true and made on information believed to be true; further that these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of any application for which it is used.

Dated: Dec 5 2006

Respectfully submitted,

By 

Dr. George N. Bennett, Ph.D.
Department Chair
Dept. of Biochemistry and Cell Biology
Rice University
Houston, TX

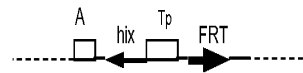
EXHIBIT A

Chromosomal integration of large designer DNA into *E. Coli*

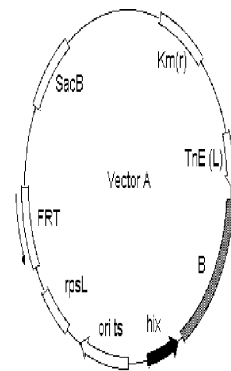


Figure 1. Components for DNA Integration

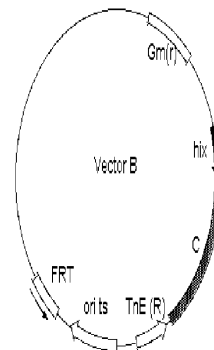
Receptor Strain



Vector A



Vector B



Integration/Excision:

Hix/hin

FRT/Flp

Tn1514- TnE(R/L)

Counter-selection:

rpsL streptomycin

sensitivity

SacB sucrose sensitivity

Antibiotic Selection:

Km kanamycin resistance

Tp trimethopim resistance

Gm gentamycin resistance

Plasmid loss selection:

ori ts temperature sensitive

replicon

Figure 2. Integration and Excision Scheme

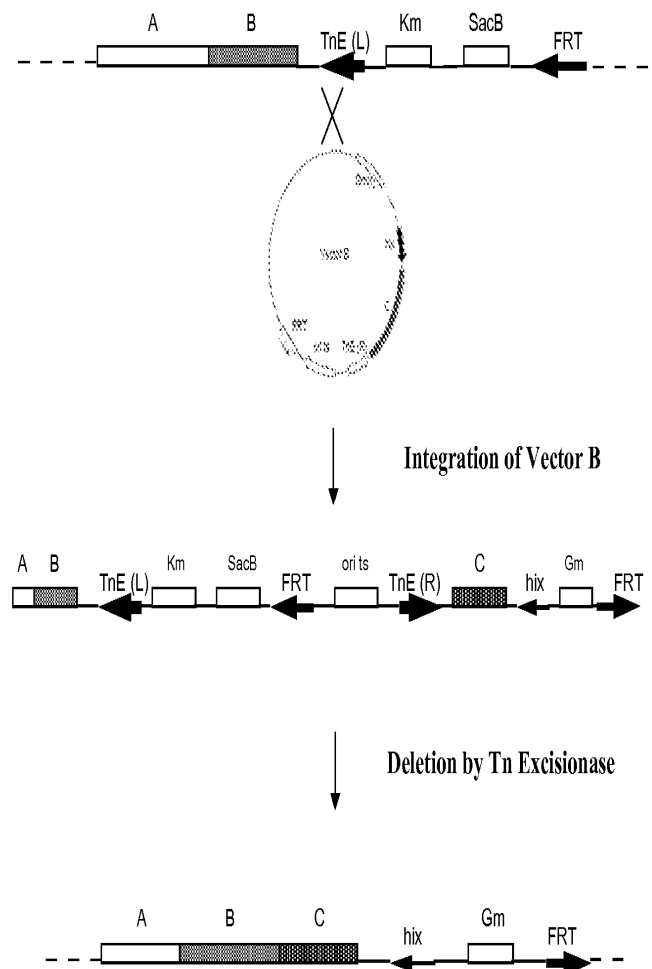
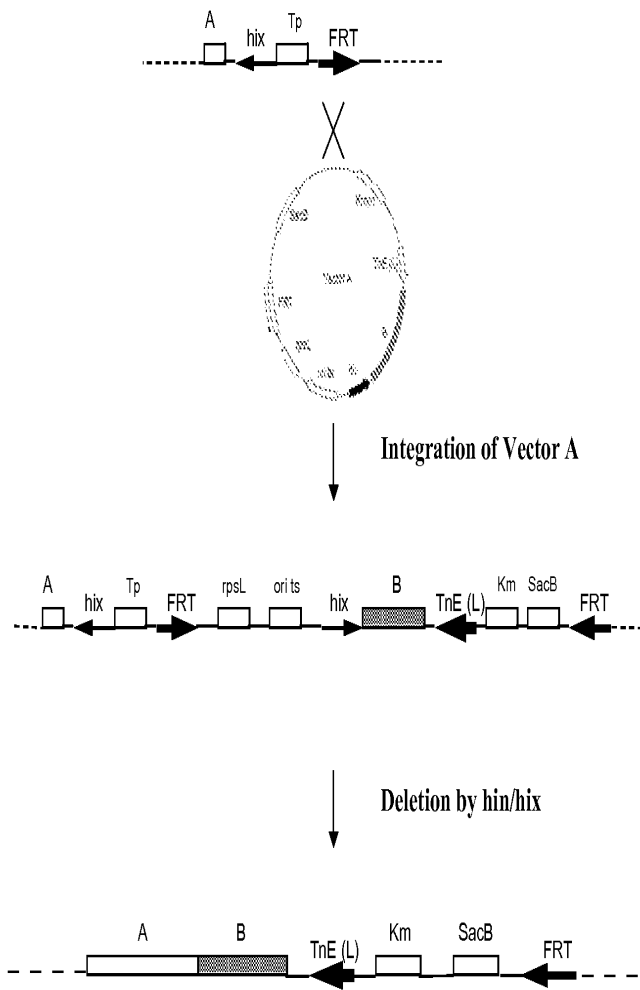


Figure 3. Construction of Receptor Strain

Susy McKay

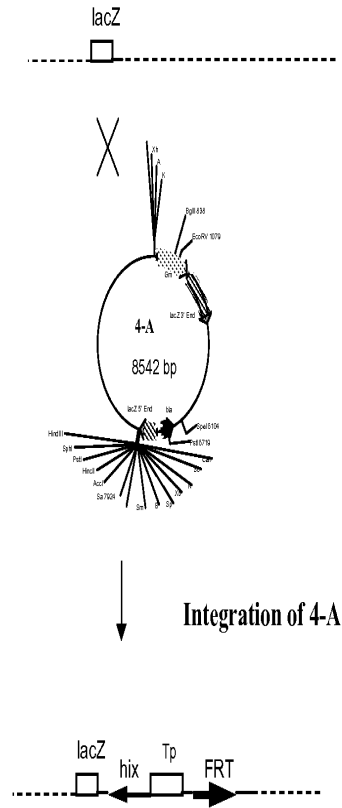
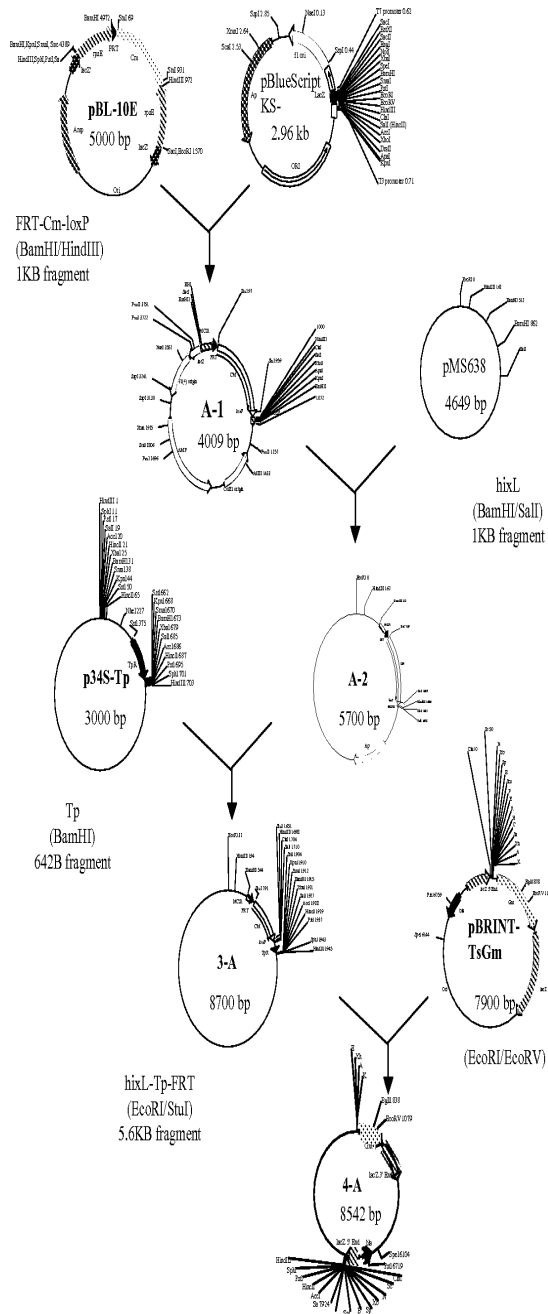


Figure 4. Construction of Vector A

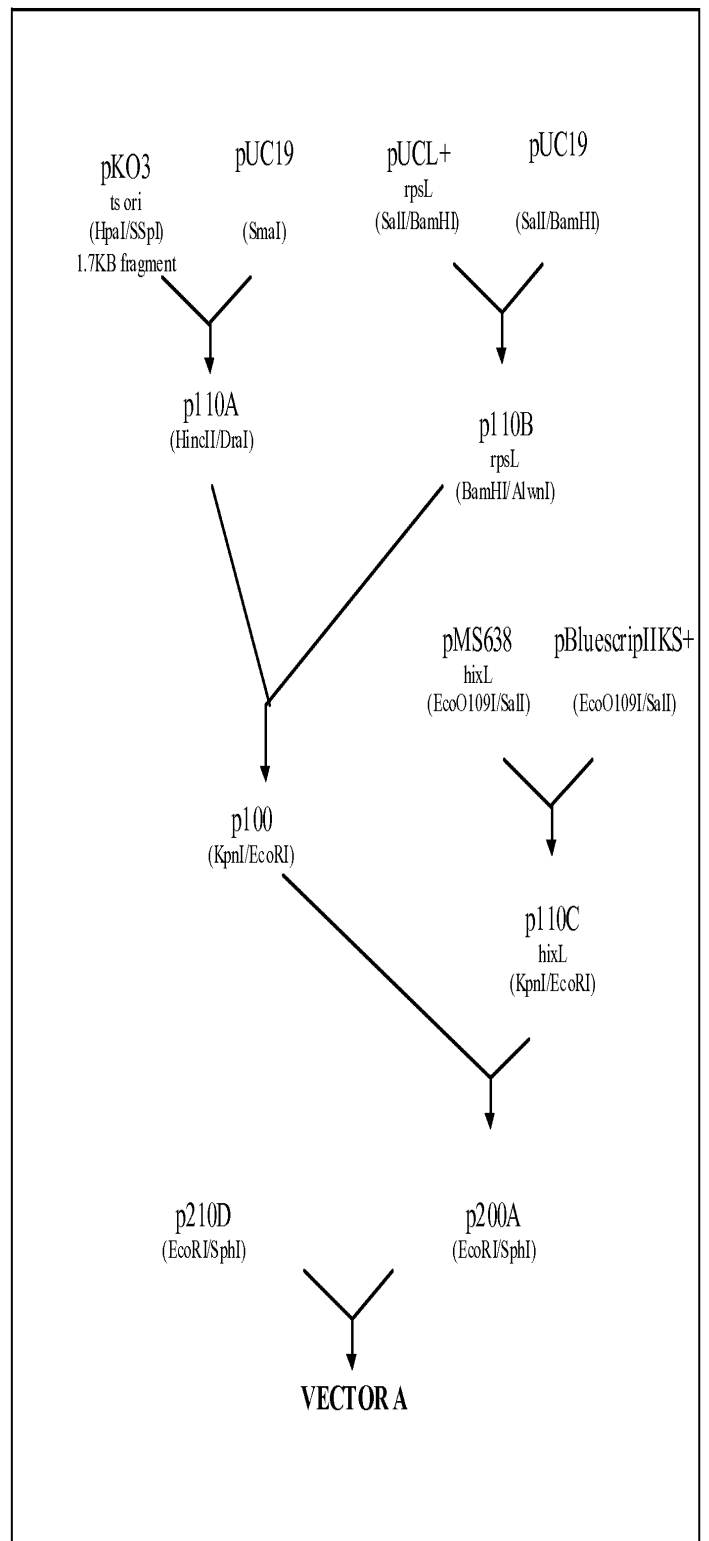
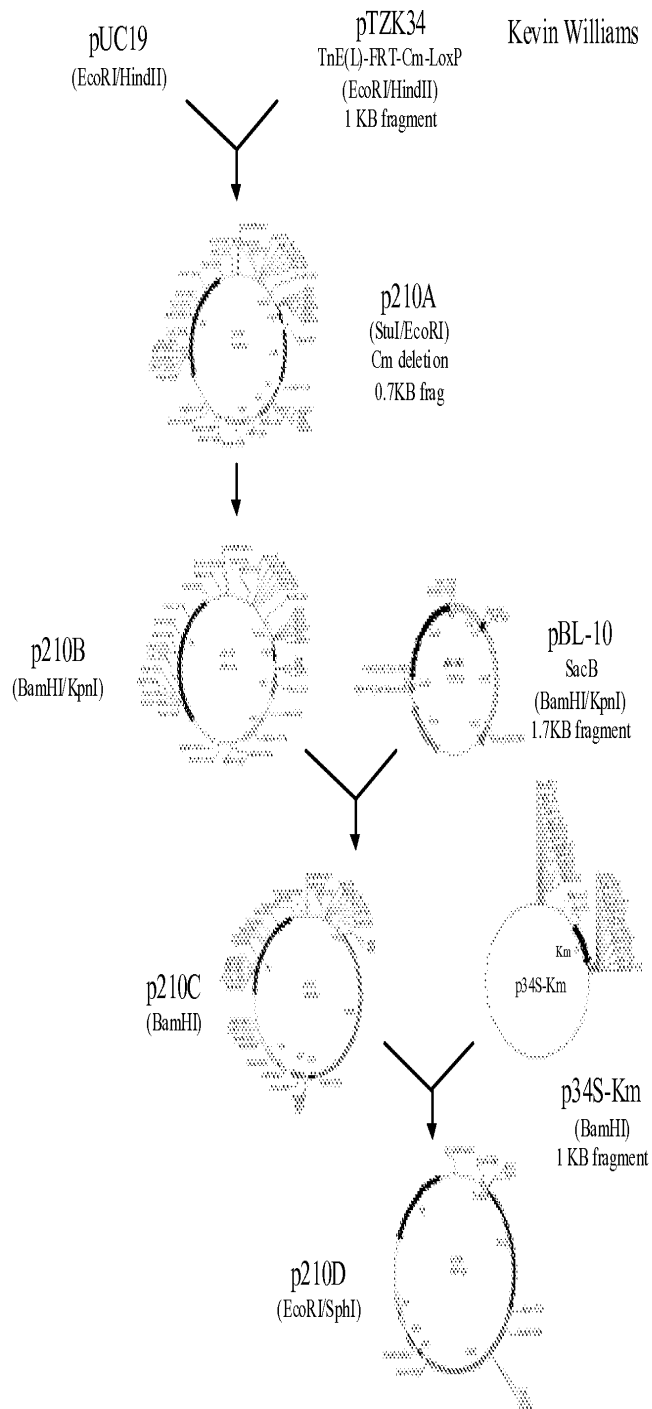


Figure 5. Construction of Vector B

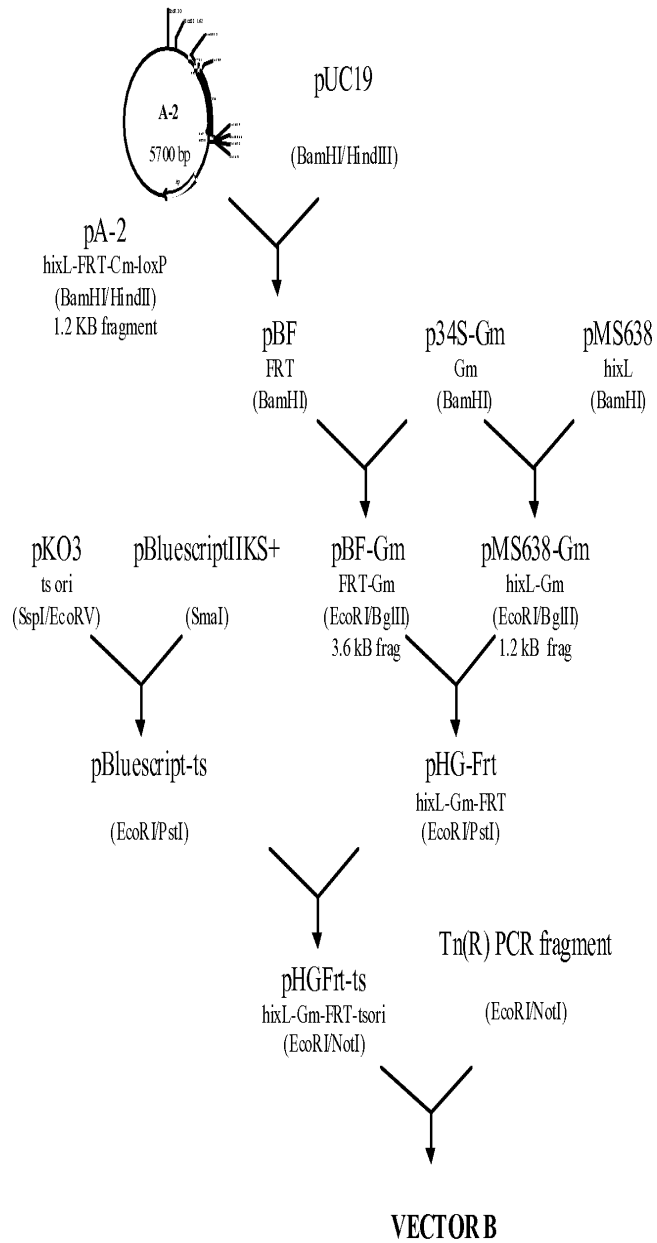
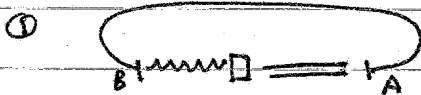


EXHIBIT B

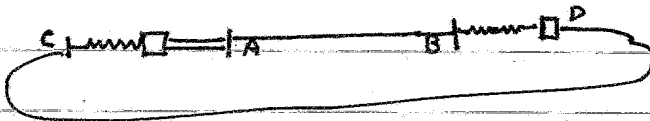


region AB cloned into plasmid ①
specific recomb site II (eg FLP yeast)

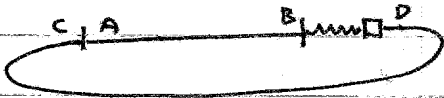
~~~~~ = conjugative Transposon seq, (eg Tn916)  
selection markers,  
replication functions as desired



② recipient plasmid or chromosome DNA  
bearing 1/2 transposon seq and II



double X results at II  
made by selection for marker =



removal of conj transposon  
precise excision (eg Tn916)  
+ selection by loss of  
gene at =

reiterate with successive  
version of ①

joining of fragments AB, CD  
at specific junction without depending  
on sequence elsewhere within segments

if use two different conj transposons  
can go with addition to  
either end & switch  
back & forth

control of FLP or transposon expression  
could be by regulation of level amount of protein  
present in host (eg by regulated expression)

SIGNATURE

George Bennett

DATE

REDACTED

DISCLOSED TO AND UNDERSTOOD BY

Liaohu Shi

DATE

WITNESS

DATE